

# Determination and Evaluation of Doses of Metronidazole in Different Quantities and Formulations with Multiple Spectroscopic Methods

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Article History:

Submitted: 22.02.2020

Revised: 01.04.2020

Accepted: 05.05.2020

## ABSTRACT

Three quick and sensitive spectrum methods have been suggested to determine Metronidazole. All spectral methods that were used included reducing Metronidazole by mixing zinc powder with hydrochloric acid. The first method (A) included the process of the conjugation of resorcinol reagent with metronidazole and the production of red chromophores, which was then estimated spectrally at a maximum wavelength of 520 nm.

Method-B including the reaction p-chloro benzaldehyde in acidic medium to product yellow color Schiff base has an absorbance at 305 nm. The method C employing a charge transfer reaction with hydroquinone acceptor in basic medium to product orange to yellow product absorbed at 335 nm. The best experimental conditions were used by applying the beer's Law for a range of concentrations 2.0-50  $\mu\text{g ml}^{-1}$ , 1.0-35  $\mu\text{g ml}^{-1}$  and 1.5-30  $\mu\text{g ml}^{-1}$  for methods A, B and C respectively, and the corresponding molar absorptivity values are  $1.375 \times 10^4$ ,  $8.672 \times 10^4$ ,  $1.995 \times 10^4 \text{ L mol}^{-1} \text{cm}^{-1}$  with a Correlation

coefficient of 0.9996, 0.9998, and 0.9988 and Limits Of Detection (LOD) 0.145  $\mu\text{g ml}^{-1}$ , 0.0776  $\mu\text{g ml}^{-1}$  and 0.110  $\mu\text{g ml}^{-1}$  and Limits of Quantitation (LOQ) 0.4412  $\mu\text{g ml}^{-1}$ , 0.2352  $\mu\text{g ml}^{-1}$  and 0.3333  $\mu\text{g ml}^{-1}$  for methods A, B and C Sequentially. The methods were successfully applied to the determination of Metronidazole in pharmaceutical preparation.

**Keywords:** Metronidazole drug, Diazotation and coupling reaction, Schiff base, Ion pair complex, Spectrophotometric methods.

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DOI: [10.31838/srp.2020.5.21](https://doi.org/10.31838/srp.2020.5.21)

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## INTRODUCTION

The chemical compound of the scientific name according to the IAUPC system, 2-methyl-5-nitroimidazole-1-ethanol (Metronidazole) can be used as an antibacterial, anti-inflammatory and antibacterial drug (Figure 1). Good reviews of pharmacokinetic and pharmacokinetic activity of Metronidazole [1] have been published. Although metronidazole properties have been studied as antimicrobials, metronidazole has been clinically tested only a few years ago. Laboratory tests have shown that the metronidazole compound has proven effective against intestinal amoebiasis in mice as well as in hepatic amoebiasis in hamsters and shows activity against Entamoeba histolytica [2,3]. Initial clinical tests have indicated that metronidazole is able to treat invasive amoebic dysentery as well as amoebic liver abscess [4]. These clinical tests have proven that metronidazole is a preferred drug in treating all forms of amebiasis in humans [5,6]. All literature has shown that Metronidazole is official in the USP. [7] and B.P. [8]. There are a number of methods for identifying recent compounds by high-performance liquid chromatography (HPLC) in different pharmaceutical preparations, [9,10] and various spectral methods have been reported for their identification [11,12]. Derived spectroscopic methods for estimating metronidazole have been reported simultaneously in different dosage forms [13,14]. High-performance chromatographic fluid injection analysis [15-18], quantitative NMR [18] quantitative magnetic resonance spectroscopy [19], and titration [20] have been described to determine metronidazole alone and with other active ingredients. Metronidazole was determined by chemical luminosity [21], electrical current measurement [22], voltmetric measurement [23], and computed tomography [24] in different forms of different pharmaceutical doses.

Most spectroscopic methods in the literature for estimating metronidazole in the visible area of the spectrum include primary reduction by primary treatment by mixing it with mineral zinc powder and hydrochloric acid [25-27] followed by diazotization. Insensitive methods include physical experiments like heating and extraction, costly reagents, also include additional removal steps. In this study, three methods of spectral measurement were developed for the quantitative calculation of metronidazole after converting to its reduced composition using a metal of zinc powder hydrochloric acid, and then, its reduced product interacts with resorcinol and then study the color product with p-chlorobenzaldehyde to form a base chef. In the third method, metronidazole reacts with hydroquinone to form a two-ion compound. The optimum conditions for this reaction and the optical, precision, and accuracy properties of the proposed validity method were studied. The proposed methods are fast and simple sensitive and they are excellent applied to estimate metronidazole in their pharmaceutical formations.

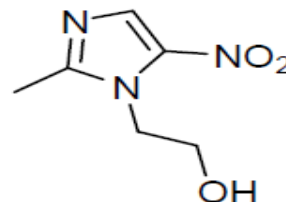


Fig.1: Chemical structure of Metronidazole

## EXPERIMENTAL

A UV-visible UV spectrophotometer detector (Shimadzu, double beam, model 2450) containing quartz cells (1 cm)

was used in the three spectral methods to estimate metronidazole in pharmaceutical preparations.

#### Chemicals and Reagents

All chemicals used were of analytical-reagent grade.

#### Resorcinol solution (0.1%)

0.1% of resorcinol solution was prepared by dissolving 0.1g of Resorcinol (99% purity) in distilled water and diluting to 100 ml in a calibrated flask.

#### Hydrochloric acid solution (5N)

In a 250 mL volumetric flask, a working solution was prepared by diluting 5N of HCl to a 2N concentration using deionized water.

#### Sodium hydroxide (5N)

In a 250 mL volumetric flask, a working solution was prepared by diluting 5N of Sodium hydroxide to a 2N concentration using deionized water.

#### A 0.1% sodium nitrite solution

A 0.1% (w/v) working solution of sodium nitrite was prepared by dissolving 0.1 g of solid in 100 ml of deionized water.

#### 5% ammonium sulfamate

5 g of ammonium sulfate solid was dissolved to prepare a working solution of 5.0% w / v and then diluted to 100 ml in volumetric flasks with deionized water.

#### P-Chlorobenzaldehyde (0.4%W/V) solution:

0.4 g of P-Chlorobenzaldehyde solid was dissolved to prepare a 0.4% w / v solution and then diluted to 100 ml in a volumetric flask with deionized water.

#### hydroquinone solution(0.1%)

0.1 g of the hydroquinone solid was dissolved to prepare a 0.1% w / v solution, then diluted to 100 mL in a volumetric flask with deionized water.

#### Preparation of the reduction Standard Solution

100 mg from the pure Metronidazole was carefully weighed and dissolved in 10 ml ethanol. 10 ml from the Metronidazole Ethanol solution was added with 1 ml 5 N HCl and 1 g of a metal of zinc powder. Zinc powder is added gradually with shaking well for an hour at 20 °C. The solution is then filtered with USA Whatman filter paper and the remaining three times washed with 10 mL Ethanol until the total volume of the filter reaches 100 mL and Ethanol. A standard working solution of 100 g / mL diluted metronidazole was prepared [11].

## THE PROCEDURES

#### The procedure of the Method A

At room temperature the standard working solution of reduced Metronidazole was transferred to titrated flasks (volumetric flasks) with a volume of 10 ml, 1 ml of 0.1%

NaNO<sub>2</sub> solution was added with good shaking for 2 minutes per 1 ml of 2 N hydrochloric. Add 2 ml of 5% ammonium sulfamate, Shake the solution for 3 minutes and add 1.2 ml of 0.1% resorcinol. After some time add a small volume of 5 N NaOH and complete the volume with ethanol up to the mark. Absorbance of red chromophores was measured at a maximum wavelength of 520 nm. A small volume (2 ml) of 5% ammonium sulfate solution is added to remove sodium nitrate in excess of the reaction need and the amount of ammonium sulfate added does not affect the color intensity of the coupling reaction.

#### The procedure of the Method B

In method B, fresh aliquots of reduced Metronidazole ranging from 0.5-2.5 ml were transferred into a series of 10 ml volumetric flasks. To each of above aliquots, 2 ml of concentrated sulfuric acid was added followed by 3 ml of p-chlorobenzaldehyde solution in methanol (0.4% w/v) and heated at 80-90° for 10 min. The absorbance of the yellow samples was measured at a maximum wavelength of 305 nm versus a blank reagent after the solution was cooled and diluted to 100 mL using ethanol where the colored solutions were stable for more than 12 hours. From the values of the standard titration curve of absorption versus drug concentration, method sensitivity was found and a precise detection limit of the concentrations used.

#### The procedure of the Method C

Into series of 10 ml volumetric flasks, transfer aliquots (0.15 -2.5 ml) of the reduced Metronidazole standard stock solution equivalent 1.5-25 µg ml<sup>-1</sup> of Metronidazole, add 1.5 ml of 0.1% hydroquinone solution, complete the volume to 10ml with ethanol and measure the absorbance of orange yellow charge transfer complexes at 335 nm against a reagent blank. A calibration graph was constructed by plotting the absorbance against the concentration of the Metronidazole drug.

#### Calibration of the pharmaceutical forms

Using the zinc powder, a quantity of the drug equivalent to 100 mg of purifying powder was reduced, after which a good grinding of 12 tablets was prepared. Then 100 ml of the pharmaceutical form was prepared for the purpose of using it in the methods used in the measurement.

#### Discuss the results

In the spectral method procedure (A) Metronidazole was estimated by reducing the nitro group to an amino group using a mixture of zinc powder and hydrochloric acid. Then metronidazole was combined with resorcinol to give the color product. Method B contains the formation of the colored Schiff base product from the reaction Metronidazole with p-chlorobenzaldehyde in acid medium. The C method indicates the occurrence of a spectral reaction in the visible region of the spectrum between Metronidazole and hydroquinone, where the transfer of electrons from Metronidazole occurs as a donor and hydroquinone as a recipient of electrons.

Optimal conditions for the reactions

A number of experiments and necessary tests have been conducted with the aim of obtaining the maximum values of sensitivity and selectivity and more stability and creating the appropriate conditions for the quantitative and qualitative formation of colored complexes.

Various parameters were first optimized, for the development of color products, univariately by systematic study of the effects of each parameter in the development of

color product. Experimental parameters suitable for the purpose of working spectral methods were installed. The maximum absorption wavelengths appropriate for each color sample were chosen from the three methods separately and measured against a sample blank. The results indicated the maximum absorbance absorbed at 520 nm, 305 nm and 335 nm for method A, B and C Sequentially as shown in figures (2-4).

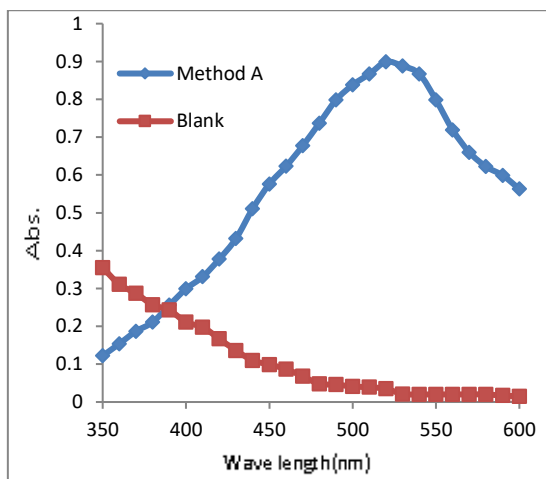


Fig 2: Absorption spectra for method A

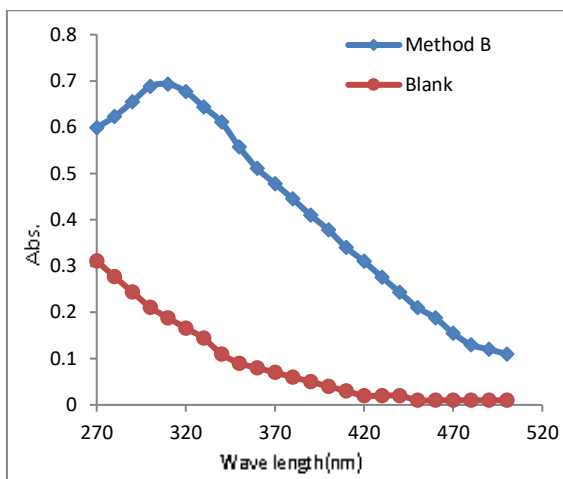


Fig 3: Absorption spectra for method B

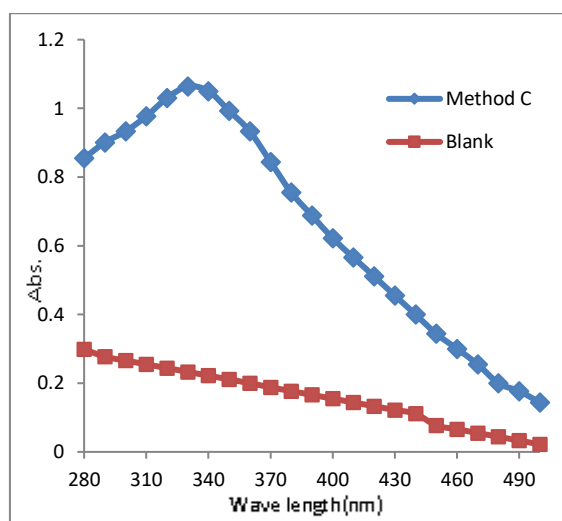


Fig 4: Absorption spectra for method C

For method A the effect of sodium nitrite concentration for diazotization was optimized in the volume range of (0.5-2 ml) (table 1). Maximum absorbance was observed when 1.0

mL of 0.1 % sodium nitrite was added and the absorption of product dye was measured at 520 nm.

Table 1: Effect of sodium nitrite volume on absorbance of azo dye in method A

Volume(ml) sodium nitrite	Absorbance (520nm)
0.5	0.946
1.0	0.9667
1.5	0.9589
2.0	0.934

The effect of many different acids on diazotization reaction was studied like, hydrochloric acid, sulfuric acid, nitric acid,

and acetic acid with 1 M of concentration. The results indicate that the HCl is suitable for users because it gives

stable diazotized product thus it was used for the subsequent experiments.

In order to develop the intensity and color of color products, different bases like sodium hydroxide, sodium carbonate, potassium hydroxide, and ammonium hydroxide were studied. The results indicate that the sodium hydroxide was suitable alkaline medium to give a maximum absorption.

Also the effect of the concentration hydrochloric acid was studied and it was found that the absorbance of the formed azo dye product was enhanced as the concentration HCl (2 N) in the range of (0.5-2 ml). The results indicate that the Maximum absorption intensity was achieved when 1 ml of 2 N hydrochloric acid was added to the diazotization reaction to give a maximum absorption as shown in (Table 2).

Table 2: Effect of hydrochloric acid volume on absorbance in method A

Volume (ml) HCl (2N)	Absorbance (520nm)
0.5	0.664
1.0	0.9667
2	0.566
2.5	0.244

The effect of pH on the reaction of method A was studied in the range (9-12) by used different volume of 5N sodium hydroxide solution .The result show that the best used in the reaction it was pH of 10.5.

The experiment of conjugation of resorcinol reagent was carried out within a range of volumes (0.4-2.0 ml) and (0.1%) with 25 µg / ml Metronidazole on the absorbance intensity of the colored azo dye and then measured the

absorbance of complex solutions with different concentrations. The result shows that 1.2 ml of resorcinol reagent solution was optimum for this method (Figure 6). The addition of 2 ml of 5% ammonium sulfamate solution was used to removed The excess of nitrite sodium on the reaction solution. An excess of ammonium sulfamate has no effect on the color intensity of the product formed.

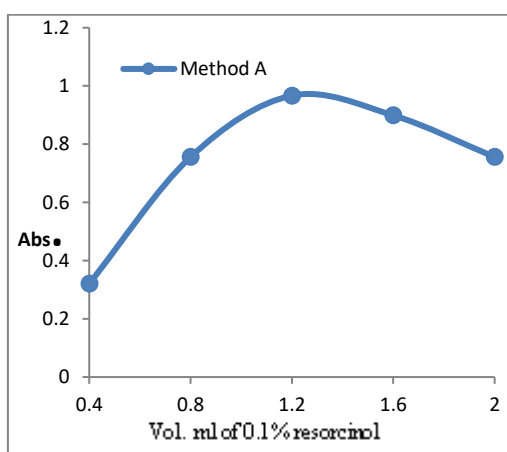


Fig 5: Effect of 0.1% resorcinol on absorbance of azo dye

For method B the optimum conditions for formation Schiff base indicated that the best volume used from concentrated sulfuric acid was 2 ml .This study done by used different

volume from concentration H<sub>2</sub>SO<sub>4</sub> in the rang (1.0-4.0 ml) as shown in table 3.

Table 3: Effect of sulfuric acid volume on absorbance in method B

Volume (ml) H <sub>2</sub> SO <sub>4</sub>	Absorbance
1	0.544
2	0.692
3	0.654
4	0.633

The influence of the quantity of p-chlorobenzaldehyde reagents on the consistency of the color expansion of Schiff base was deliberate by, standardize the absorbance of the solutions containing a stable concentration of 20 µgml<sup>-1</sup>of

the Metronidazole drugs and assorted rate of the p-chlorobenzaldehyde reagent. It was found that 3.0 ml of (0.4% w/v) p-chlorobenzaldehyde was sufficient for maximum absorbance as shown in figure 6.

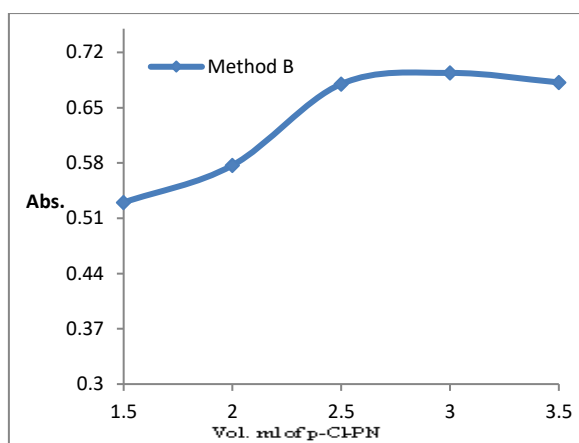


Fig 6: Effect of 0.4% P-Cl-PN on absorbance of Method B

In method C the effect of acceptor hydroquinone concentration on the intensity of the color ion pair complex was development and tested by using different volume of hydroquinone reagent in the range (0.5–2.5 ml), and a fixed

concentration  $15 \mu\text{g ml}^{-1}$  of the Metronidazole drugs. The result shows that 1.5 ml of 0.1% hydroquinone solution was optimum for maximum absorbance in basic solution (pH=8.5) as show in table 4.

Table 4: Effect of hydroquinone volume on absorbance in method C

Volume (ml)	Absorbance (335nm)
0.5	0.439
1	0.776
1.5	1.065
2	1.021
2.5	0.989

According to the results obtained upon studying the effect of different orders of addition on the absorption of the colored products was studied. The results are shown in Table (5) indicate that the addition [Metrodiazol + HCL+NaNO<sub>2</sub>+R+NaOH] give a higher absorption of

colored product for method A and [Metrodiazol+P-CL-PN + H<sub>2</sub>SO<sub>4</sub>] for method B and [Metrodiazol + Hydroquinone+ NaOH ] for method C. So it was adopted in subsequent experiments.

Table 5: Effect of Sequins of addition

Method	Sequins of addition	Absorbance
Method A	Metronidazole +HCL+NaNO <sub>2</sub> +R+NaOH	0.998
	Metronidazole +HCL+R+NaNO <sub>2</sub> +NaOH	0.887
	Metronidazole +HCL+R+NaOH+NaNO <sub>2</sub>	0.665
	Metronidazole +HCL+NaNO <sub>2</sub> +NaOH+R	0.982
Method B	Metronidazole +P-CL-PN+H <sub>2</sub> SO <sub>4</sub>	0.694
	Metronidazole +H <sub>2</sub> SO <sub>4</sub> + P-CL-PN	0.689
	P-CL-PN+H <sub>2</sub> SO <sub>4</sub> +Metrodiazol	0.690
Method C	Metronidazole +Hydroquinone +NaOH	1.067
	Hydroquinone+ Metrodiazol +NaOH	1.001
	Metronidazole +NaOH +Hydroquinone	0.805

R= resorcinol , P-CL-PN= p-chloropenzaldehyde  
In method A the maximum time required for coupling reaction to be completed was found to be 10 min. at room temperature. After that period, In Method B, the effect of time was studied as no change in absorption values was observed when changing the time from 30 minutes to 12 hours at a temperature of 90 degrees Celsius. In method C, the effect of time on absorbance values was studied to

ascertain the ion-pair complex. At 20 ° C and vibration speed for 2.0 minutes, the maximum constant absorption value was reached. Ion-pairs complex was stable for 24 hours There was no change in the intensity of the color. Changes in time values do not affect the analytical values, as this confirms that the reaction is rapid and that the coloring product formed does not degrade.

Methods Validation

Under the optimum conditions for the three methods, the linearity was checked and the calibration curves were constructed between the absorbance and corresponding concentration at the selected wavelength for each method. The linearity were found to be valid in the range 2.0-50µg/ml,1.0-35µg/ml and 1.5-30 µg/ml for methods A, B and C respectively as shown in Figure (7).

Excellent calibration curve values, represented by correlation coefficient R, very small Y-intercept values, regression coefficient, molar absorptivity values, Sandell sensitivity values, detection limits, and quantitative measurement for each of the three methods were obtained, in addition to calculating the values of statistical quantities according to ICH data [28]. Table 6 shows that.

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated(29) according equations (1) and (2):

**L.O.D. = 3σ/S ..... (Eq. 1)**

**L.O.Q. = 10 σ/S ..... (Eq. 2)**

**Where σ = standard deviation of intercept (n=5).**

**S=slope of linearity .**

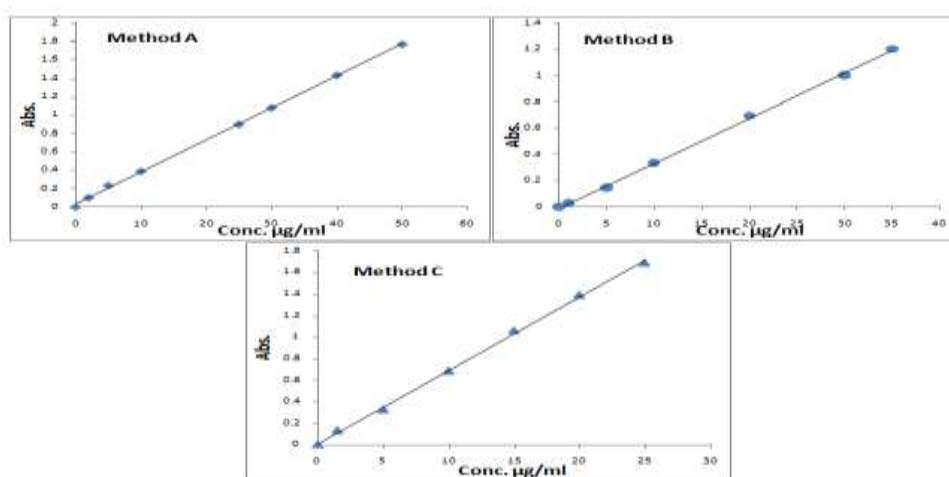


Fig 7: Linearity curves for methods A,B,C and D

Table 6: Analytical parameter of methods A,B and C

Parameters	Method A	Method B	Method C
λmax. (nm)	520	305	335
Beer's law limits (µg/ml)	2.0-50	1.0-35	1.5-25
R2Correlation coefficient (r)	0.9996	0.9998	0.9988
Intercept	0.033	0.008	0.010
Slope (b)	0.068	0.034	0.030
Limits Of Detection LOD	0.145	0.0776	0.110
Limits Of Quantitation LOQ	0.4412	0.2352	0.3333
Molar absorption (L/ mol cm)	1.375×10 <sup>4</sup>	8.672×10 <sup>4</sup>	1.995×10 <sup>4</sup>
Sandell's sensitivity (µg/cm <sup>2</sup> )	0.475461×10 <sup>-3</sup>	0.09.2007×10 <sup>-3</sup>	0.267325×10 <sup>-3</sup>

Accuracy and precision

Accuracy and precision were studied by measuring absorption at 525 nm, 305 nm and 335 nm for methods A, B and C with different concentrations of the Metronidazole pure drug within the limits of Beer's law, the average recovery (99.70-101.00%), (99.45-107.00%) and (99.50-100.10%) for each method indicate that the methods is of high accuracy and precision [30]. Table 7 shows all the

results obtained. Three ranges of concentrations were analyzed within three replicates.

The values of (RSD%) and the relative error (RE%) were calculated for all concentrations that were worked on. The results obtained showed an excellent compatibility with the standard values and this indicates the success of the methods used in this analytical study.

Table 7: Statistical values for accuracy and precision

Method	Conc. of Metronidazole ( $\mu\text{g.mL}^{-1}$ )		Recovery%	E %	RSD %
	Taken	Found*			
Method A	5	5.05	101.00	1.00	1.142
	10	9.97	99.70	0.30	1.550
	20	19.99	99.95	0.05	1.996
Method B	5	5.03	100.60	0.60	1.109
	10	10.07	100.70	0.70	1.570
	20	19.89	99.45	0.55	2.499
Method C	5	4.99	99.80	0.20	1.006
	10	9.95	99.50	0.50	1.899
	20	20.02	100.10	0.10	2.033

\*Average of three determinations.

#### Effect of interferences

In order to test the efficiency and selectivity of the proposed method, the effect of some foreign substances that usually present in dosage forms was studied under the optimum conditions of each methods. The results showed that there is no interferences at their regularly added levels.

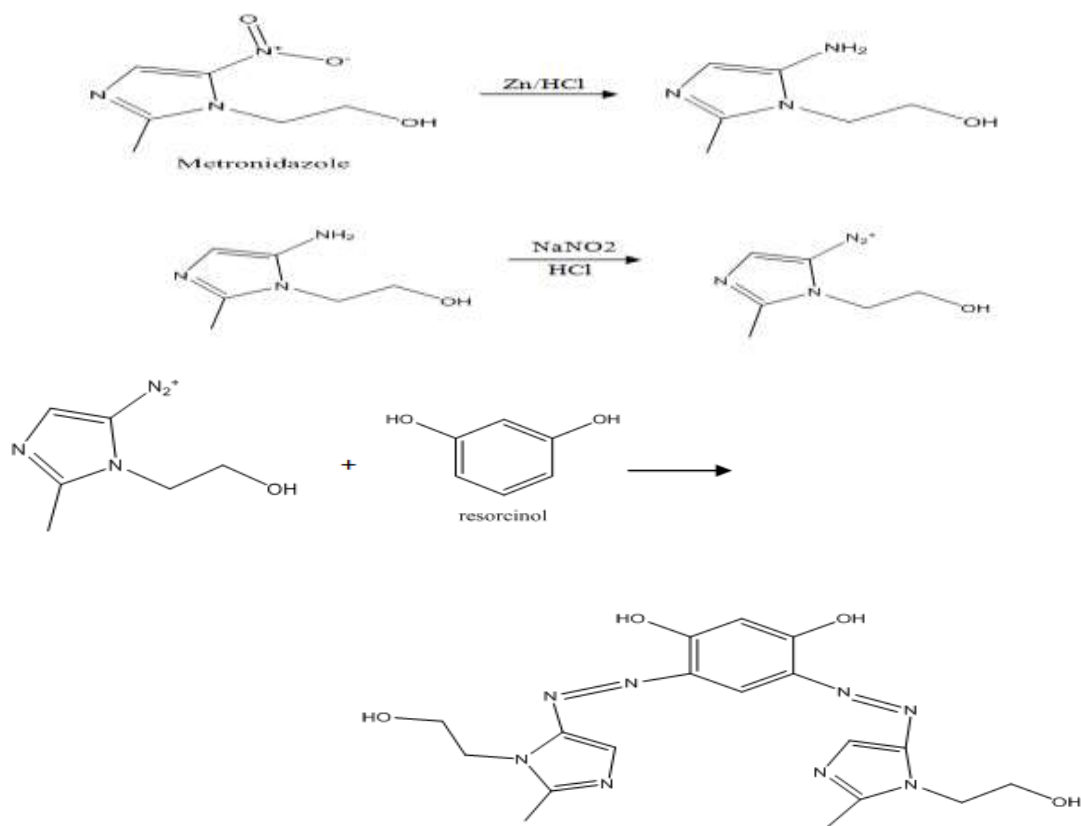
#### The stoichiometry of the reaction

The Job's method for standard deviation was used in the evaluation of the three spectral methods in estimating Metronidazole, as different ratios of each reagent were

mixed with Metronidazole to reach a fixed volume in all measurements [31].

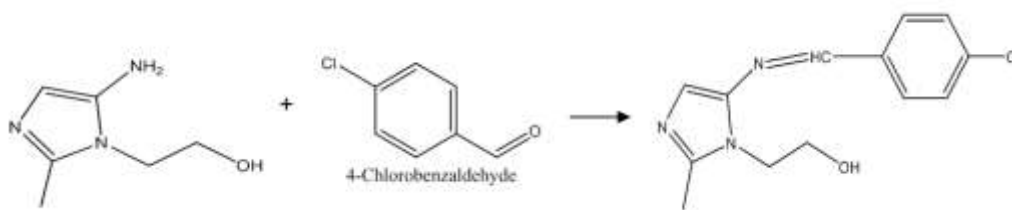
In all the methods under study, a range of volumes (1 – 9 ml) of Metronidazole was used, and a range of reagent volumes (1 – 9 ml) of the reagent. The absorbance was measured at 520 nm, 305 nm and 335 nm methods A, B and C respectively against the blank reagent. The results) show that the ratio is 1:1.

The suggested reactions pathway for the reaction product according to that we can explain the mechanism of the methods in scheme 1, 2 and 3 for method A, B and C Sequentially.

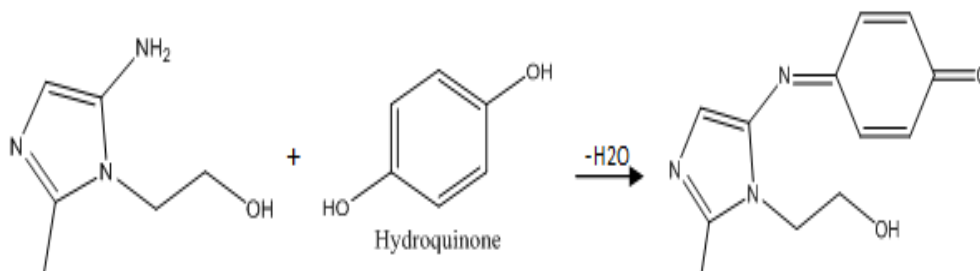


Scheme 1: The proposed pathway to the reaction for method A





Scheme 2: The proposed pathway to the reaction for method B



Scheme 3: The proposed pathway to the reaction for method C

In method C, the amine group in the reduced metronidazole shows a wide participation with the added reagents.

#### Determination of the stability constant

The stability constant ( $K_f$ ) of the reaction products are calculated according to the follow equation [32]:

$$K_f = \frac{(A / A_m)}{[1 - A / A_m] n^{-1} C^n n^n}$$

Where: A = maximum absorbance of the continuous variation curve Figure 6,  $A_m$  = absorbance corresponding to intersection of two tangents of the continuous variation curve, n = number of molecules of the reagent in the

reaction product, C = molar concentration of the drug and  $K_f$  = formation constant of the complex.

The stability constant of the reaction products of methods A,B, and C were  $61.7997 \times 10^3 \text{ liter} \cdot \text{mol}^{-1}$ ,  $19.2261 \times 10^3 \text{ L} \cdot \text{mol}^{-1}$ , and  $11.3813 \times 10^3 \text{ L} \cdot \text{mol}^{-1}$  respectively.

The Gibbs free energy change of the reaction ( $\Delta G$ ) [33] was also calculated adopting the following equation:

$$\Delta G = - 2.303 R T \log K_f$$

Where:  $\Delta G$  = Gibbs free energy change of the reaction (kJ.  $\text{mol}^{-1}$ )

R = Universal gas constant (8.314 joules)

The higher  $K_f$  and  $\Delta G$  values obtained indicate very stable reaction products. The results were summarized in table 8.

Table 8: Stability Constance and Gibbs free energy values for methods

Constants	Method A	Method B	Method C
$K_f(\text{L} \cdot \text{mol}^{-1})$	$61.7997 \times 10^3$	$19.2261 \times 10^3$	$11.3813 \times 10^3$
$\Delta G(\text{kJ} \cdot \text{mol}^{-1})$	-21.8326	-19.5217	-23.4117

#### Analytical application

When comparing the obtained statistical results, it was found that there is a close agreement between the values of the results obtained by the proposed methods and the values of the announced tables 9 [34]. The statistical values (t-test) from (F-test) in order to obtain the most significant indications at the 95% confidence level, we find that the calculated t and F values did not exceed the tabulated values (t = 2.77) and (F = 9.39), which indicates that the proposed

methods This study is accurate and precise, as shown in Table 9.

The results in table 9 shows low RSD percentage (<1%) which referred to good precision, and recovery precision range (104.40, 99.40), (98.40-100.70), (100.05-102.20) which referred to good accuracy showed there are no interaction of the excipients and the good sensitivity of the method signed to capable applied the developing methods successfully to determination of Metronidazole in pharmaceutical preparation.

Table 9: Determination of Metronidazole in Formulations preparation

Proposed methods	Conc. ( $\mu\text{g/ml}$ )	Commercial Formulations analyzed					
		FLATRO (tablet 200 mg)			KINDAZOL (Tablet 500 mg)		
	Taken conc. ( $\mu\text{g/ml}$ )	5	10	20	5	10	20



Method A	Found conc. (µg/ml)	5.02	9.95	19.88	5.05	9.99	19.98
	Recovery %*	100.40	99.50	99.40	101.00	99.90	99.90
	RSD%*	0.68	0.94	0.66	0.83	1.01	0.99
Reference method	Recovery% ± SD**	101.27±0.06			100.06±0.09		
Method B	Taken conc. (µg/ml)	5	10	20	5	10	20
	Found conc. (µg/ml)	4.92	10.07	20.08	5.03	10.02	20.06
	Recovery %*	98.40	100.70	100.40	100.60	100.20	100.30
	RSD%*	0.59	0.95	0.88	0.91	0.87	0.96
Reference method	Recovery% ± SD**	103.55±0.07			102.22±0.05		
Method C	Taken conc. (µg/ml)	5	10	20	5	10	20
	Found conc. (µg/ml)	5.11	10.05	20.04	5.06	10.08	20.01
	Recovery % *	102.20	100.50	100.20	101.20	100.80	100.05
	RSD%*	0.87	0.90	0.95	1.07	0.83	0.69
Reference method	Recovery% ± SD**	104.55±0.05			103.04±0.11		

\*Average of three determination, \*\* Average of five determination, RSD = Relative standard deviation, SD = standard deviation

## CONCLUSIONS

All the spectral methods suggested in Metronidazole estimation were simple, precise, accurate, and highly sequential. The method A depended on the diazotization reaction coupling to formed azo dye with resorcinol reagent to produced color azo dye absorbed at 520 nm. Method B containing reaction between the Metronidazole drug with p-chloropenzaldehyde to form color product absorbed at 305 nm and method C depend on formation colored ion pair complex by reaction of Metronidazole with hydroquinone absorbed at 335 nm. The advantage of this procedures were does not need to the control of temperature control, solvent extraction and simple for used also its accrue and sensitive methods. A color interaction does not require difficult conditions or a complex reagent. The stability of color samples for more than 12 hours was the reason for obtaining excellent analytical values and results. Also, it does not require any pre-manipulations of the models taken for measurement. From this we conclude that all the data extracted in the manuscript for all methods of spectroscopy in estimating pure metronidazole. The proposed methods are precise, linear, and therefore can be used for routine analysis of metronidazole, in the pharmaceutical industry, and for research.

## THANKFULNESS

I would like to commend the University of Basra, College of Pharmacy, Department of Pharmaceutical Chemistry, for my support in completing this manuscript.

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